

## UPDATE ON THE CLINICAL DEVELOPMENT OF CANDIDATE MALARIA VACCINES

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**Abstract.** The recent availability of significantly increased levels of funding for unmet medical needs in the developing world, made available by newly created public-private-partnerships, has proven to be a powerful driver for stimulating clinical development of candidate vaccines for malaria. This new way forward promises to greatly increase the likelihood of bringing a safe and effective vaccine to licensure. The investigators bring together important published and unpublished information that illuminates the status of malaria vaccine development. They focus their comments on those candidate vaccines that are currently in or expected to enter clinical trials in the next 12 months.

### INTRODUCTION

The development of a safe and effective malaria vaccine remains an urgent unmet medical need for vast populations living in malaria-endemic regions. Because severe morbidity and death due to *Plasmodium falciparum* disproportionately occurs in infants and young children living in sub-Saharan Africa, this target population has been the principal focus of malaria vaccine development. Recently, significantly increased funding through private and public partnerships has encouraged the industrial sector to become more involved in the development of candidate malaria vaccines. This process in turn has led to more clearly articulated target product profiles and clinical development plans that have facilitated decision-making and promoted rapid progress on several fronts. This review will update the status of development of malaria vaccine development, focusing in particular on those candidate vaccines that will soon be or are currently in clinical trials (Figure 1). A summary of the vaccines discussed in this review is shown in Table 1. All volunteers were recruited using non-coercive means under clinical protocols and informed consent documents reviewed and approved by local and region ethical review boards.

### PRE-ERYTHROCYTIC VACCINES

Pre-erythrocytic vaccines are designed to target sporozoites or schizont-infected liver cells and thus prevent the release of primary merozoites from infected hepatocytes. Preclinical studies in rodents and humans immunized with radiation-attenuated sporozoites indicated that this may be achieved by antibodies that target sporozoites and block their ability to infect liver cells or by cell-mediated responses that kill parasite-infected hepatocytes before they can release infectious merozoites.<sup>1,2</sup> Relative to the number of circulating blood stage parasites that result in clinical disease, the number of sporozoites or liver stage schizonts is quite small; however, the window of opportunity to interrupt the pre-erythrocytic stage of the life cycle is rather brief. Protective responses against pre-erythrocytic stages must be 100% effective to

translate into sterile immunity in non-immune individuals. Protective responses that are 80–90% effective may have biologically meaningful consequences: thus, a two-day delay in the time to patent parasitemia has been estimated to reflect an approximately eight-fold reduction in the number of infectious sporozoites that develop into liver stage schizonts or the number of infectious merozoites released from infected liver cells. Whether a substantial reduction in initial parasite load will translate into a medically significant impact on the occurrence of clinical or severe disease in an endemic setting is unclear, but this is one possible mechanism by which insecticide-treated bed nets may act to reduce severe malaria morbidity.<sup>3</sup> Indirect evidence from vaccines studies is limited; however, a field trial of one pre-erythrocytic vaccine candidate (RTS,S/AS02A), which indicated significant protection from infection over two seasons, also showed a promising trend towards reducing episodes of clinical malaria during the same period.<sup>4</sup>

The circumsporozoite (CS) protein is the pre-erythrocytic antigen against which immune responses are most clearly

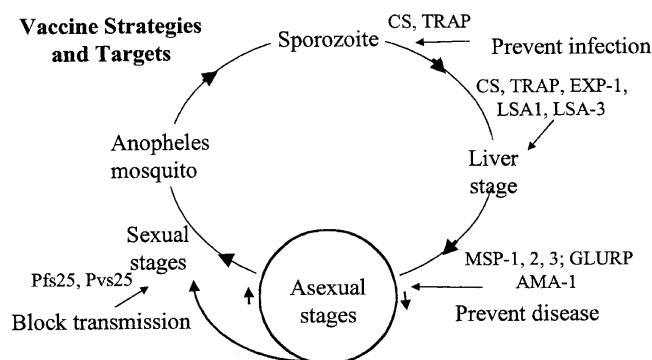


FIGURE 1. Malaria life cycle and vaccine targets. CS = circumsporozoite; TRAP = thrombospondin-related adhesive protein; EXP-1 = exported antigen 1; LSA1 = liver stage antigen 1; MSP-1 = merozoite stage protein 1; GLURP = glutamate-rich protein; AMA-1 = apical membrane antigen 1; Pf = *Plasmodium falciparum*; Pv = *P. vivax*.

TABLE 1  
Candidate malaria vaccines currently in clinical development\*

	Pre-erythrocytic vaccines					
	Research	Preclinical development	Phase 1a	Phase 2a	Phase 1b	Phase 2b
<b>CSP</b>						
RTS,S/AS02A (CSP/HBsAg particle in AS02A adjuvant) (GSK)	X	X	X	X	X	X
HBcAg-CSP VLP (Malarivax Apovia)	X	X	X	X		
Modified Vaccinia Ankara (MVA) CSP tested in combination with RTS,S, SA02 (Oxford-GSK)	X	X	X	X		
Long synthetic CSP peptide (Lausanne)	X	X	X			
CSP DNA immunization (Vical/NMRC)	X	X	X	X		
Long Vivax CSP C-terminus 72 amino acid synthetic peptide (MVDC)	X	X	X			
Long Vivax CSP N-terminus 77 amino acid synthetic peptide (MVDC)	X	X	X			
Long Vivax CSP repeat 48 amino acid synthetic peptide (MVDC)	X	X	X			
<b>Other antigens</b>						
Long synthetic LSA-3 peptide (Pasteur Institute)	X	X	X			
<i>Lactococcus lactis</i> recombinant LSA3 (Full length) in AS02 adjuvant (Pasteur Institute)	X	X	X	X		
Lipopeptide LSA3 without adjuvant (Pasteur Institute)	X	X	X	X		
Modified Vaccinia Ankara (MVA) CSP + LSA-1 epitope (Oxford) only a phase 1 with MVA. Combination noted above	X	X	X			
Fowl Pox 9 CSP + LSA-1 epitope (Oxford)	X	X	X			
<i>Escherichia coli</i> LSA-1 recombinant protein (WRAIR)	X	X				
MVA prime-boost DNA Multi-epitope string + TRAP (Oxford)	X	X	X	X	X	X
MVA prime-boost Fowl Pox 9 Multi-epitope string + TRAP (Oxford)	X	X	X	X	X	
<b>Asexual stage vaccines</b>						
<b>MSP-1</b>						
Recombinant full length MSP-1 3D7 + FCB1 (Heidelberg/WRAIR)	X	X				
<i>E. coli</i> expressed recombinant protein MSP-1 42-kd 3D7 (FMP-1 WRAIR)	X	X	X	X	X	
<i>E. coli</i> expressed recombinant protein MSP-1 42-kd FVO (WRAIR)	X	X				
<i>Pichia pastoris</i> expressed recombinant protein MSP-1 42-kd 3D7 + FVO (MVDU)	X	X				
Baculovirus recombinant protein MSP-1 19-kD (Pasteur Institute)	X	X				
Baculovirus recombinant protein MSP-1 42-kD FUP (U of Hawaii/Antigenics)	X	X				
<i>P. pastoris</i> recombinant AMA-1 and MSP-1 chimera (SMMU)	X	X	X			
<b>Other MSPs</b>						
Long synthetic peptide MSP-2 (Lausanne)	X	X				
Long synthetic peptide MSP-3 (Pasteur Institute)	X	X	X		X	
<i>L. lactis</i> MSP-3 recombinant (Pasteur Institute)	X	X	X			
MSP-3-GLURP hybrid vaccine (SSI)	X	X				
<b>AMA-1</b>						
<i>E. coli</i> expressed recombinant protein AMA-1 3D7+FVO (MVDU)	X	X	X			
<i>P. pastoris</i> expressed recombinant AMA-1 protein (BPRC) Doubt	X	X				
<i>E. coli</i> recombinant protein AMA-1 3D7 (FMP-2.1 WRAIR)	X	X				
<b>Other proteins</b>						
Long synthetic GLURP peptide (SSI)	X	X	X			
<b>Transmission-blocking vaccines</b>						
<i>Saccharomyces</i> recombinant protein PvS25 (MVDU)	X	X	X			
<i>P. pastoris</i> recombinant protein PfS25 (MVDU)	X	X				
<b>Combination (multistage) vaccines</b>						
Multi-stage pre-erythrocytic DNA vaccination (Vical/NMRC)	X	X	X			
Recombinant FMP-1 plus RTS,S MSP-1 3D7 + CSP (WRAIR)	X	X	X	X		

\* CSP = circumsporozoite protein; HBsAg = hepatitis B surface antigen; GSK = GlaxoSmithKline; HBcAg = hepatitis B core antigen; VLP = virus-like particle; NMRC = Naval Medical Research Center; MVDC = Malaria Vaccine Development Center; LSA = liver stage antigen; WRAIR = Walter Reed Army Institute of Research; TRAP = thrombospondin-related adhesive protein; MSP = merozoite surface protein; FMP = Falciparum merozoite protein; MVDU = Malaria Vaccine Development Unit; AMA = apical membrane antigen; SMMU = Second Military Medical University; GLURP = glutamate-rich protein; SSI = Statens Serum Institute; BPRC = Biomedical Primate Research Center; Pv = *Plasmodium vivax*; Pf = *P. falciparum*.

linked to protection. This sporozoite membrane-associated protein has been very well characterized in terms of primary structure and variability across a large number of parasite strains. Antibodies are primarily directed against the central conserved repeat region, with minor B cell epitopes mapped to non-repeat flanking regions. These flanking regions include short, highly conserved regions interspaced with variable sequences in which lie a majority of the effector CD4+ and CD8+ epitopes identified to date. Little is known about how CS is folded in its native state, but there appear to be at least two C-C bonds formed within or between CS molecules.

Numerous vaccine constructs have been developed that are directed against the CS protein. The existence of a safe and

reliable human challenge model that incorporates a standardized sporozoite challenge has revealed that the most promising CS candidates include immunodominant B cell epitopes present in the central repeat (NANPx) region and T cell epitopes from the C terminal portion of the molecule.<sup>5-7</sup> These studies also emphasize the importance of delivering these epitopes using strategies that maximize both humoral and cellular responses. Non-human primate models, especially rhesus monkeys, have been useful in identifying optimally immunogenic vaccine formulations and for conducting preclinical safety studies.

**RTS,S/AS02A.** Currently, the malaria vaccine candidate for which clinical development is most advanced is RTS,S/

AS02A made by GlaxoSmithKline Biologicals (GSKBio) (Rixensart, Belgium). This pre-erythrocytic stage vaccine is based on the CS protein of the 3D7 clone of *P. falciparum*. The components of this vaccine are two polypeptides (RTS and S) that are expressed simultaneously in *Saccharomyces cerevisiae*. RTS is a single polypeptide chain corresponding to amino acids 207-395 of the CS protein fused to the amino terminus of the hepatitis B surface antigen (HBsAg; adw serotype). S is a polypeptide of 226 amino acids that corresponds to HBsAg. Each RTS molecule includes 19 copies of the tetrapeptide repeat motif (NANP) fused to the C-terminal region of the protein (minus the hydrophobic anchor sequence). During purification, the two polypeptides spontaneously assemble to form composite particulate structures (RTS,S) that constitute the vaccine antigen.<sup>6</sup>

The preclinical and early clinical development of RTS,S was conducted by GSKBio in collaboration with the Walter Reed Army Institute of Research<sup>8</sup> (WRAIR) (Washington, DC) where it was demonstrated that RTS,S formulated with the proprietary adjuvant SBAS2 (now renamed AS02A) was superior in terms of immunogenicity and efficacy against experimental sporozoite challenge than was RTS,S formulated with less potent adjuvants.<sup>7</sup> The current presentation involves a two-vial system including lyophilized antigen (RTS,S) and a vial or syringe of sterile AS02A adjuvant that are mixed just prior to injection. AS02A consists of an oil-in-water emulsion that incorporates the immunostimulants monophosphoryl lipid A and the saponin derivative QS21. The formulation induces high levels of CS repeat-specific antibodies and stimulates Th-1 cellular immune responses characterized by antigen-specific production of interferon- $\gamma$  (IFN- $\gamma$ ). These responses are presumed to be an important component of the protection observed in clinical trials.<sup>9</sup> The vaccine also induces very high levels of antibodies against HBsAg.

Phase 2 safety and immunogenicity trials of RTS,S/AS02A in malaria-naïve and malaria-immune subjects have confirmed that the vaccine is safe and highly immunogenic. Experimental challenge studies have shown that the vaccine confers sterile immunity in approximately 50% of volunteers when it is administered on a two- or three-dose schedule.<sup>10</sup> Moreover, compared with non-immunized controls, many RTS,S/AS02A-immunized volunteers who have not been completely protected demonstrate significantly prolonged (24–48 hours or more) prepatent periods compared with simultaneously challenged unimmunized controls. However, protective immunity conferred by this vaccine wanes over time and a minority of initially protected subjects remain protected when rechallenged up to six months after the first challenge.<sup>11</sup>

The clinical development plan for RTS,S has as its objective the prevention of severe malaria disease in infants and children in sub-Saharan Africa. The pediatric clinical development program has been conducted under a partnership between GSKBio and the Malaria Vaccine Initiative (MVI). The evaluation of this vaccine in the field began with a Phase 1 safety and immunogenicity study in malaria-experienced adults. This study was conducted by investigators at the Medical Research Council (MRC) laboratories in The Gambia and demonstrated that the vaccine was safe and immunogenic.<sup>12</sup>

Subsequently, a randomized, double-blind, controlled Phase 2b pilot efficacy study was conducted in 360 malaria-experienced adult Gambian men who were randomized to

receive three doses of either RTS,S/AS02A or rabies vaccine given intramuscularly at 0, 1, and 6 months.<sup>4</sup> The vaccination schedule was timed to coincide with the dry season during which transmission of *P. falciparum* was low. The final dose was administered approximately one month before the expected onset of the rainy season. Volunteers were treated with sulfadoxine-pyrimethamine to clear asexual-stage parasites one month before the beginning of the 15-week efficacy follow-up period. The vaccine was safe, mildly reactogenic, and immunogenic. In this trial, the primary endpoint was time to infection. Vaccine efficacy was calculated from the adjusted hazard ratio estimated from the Cox regression. Efficacy was 34% overall (95% confidence interval [CI] = 8–53%,  $P = 0.014$ ), but protection against infection was estimated to be 71% (95% CI = 46–85%) during the first nine weeks of follow-up, and 0% during the next and final six weeks of the follow-up period. Subset analysis showed that younger, less malaria-experienced men became infected in the control group earlier than did the older, more highly immune members of their cohort. As vaccine efficacy waned, the incidence in the RTS,S/AS02A group was being compared with the control group from which the most susceptible subjects had been removed. Antibody responses also declined during the period of follow-up such that the mean levels at the end of the follow-up period were equivalent to those after a single dose of vaccine.

**Recent clinical development of RTS,S/AS02A in pediatric populations.** Encouraged by these results, the GSKBio/MVI partnership sponsored two Phase 1 safety and immunogenicity pediatric trials conducted by the MRC in Upper Basse, The Gambia. An initial safety study in children 6–11 years old was followed by a second study in children 1–5 years old. In each, a double-blind, randomized, controlled, staggered dose-escalation design was used to evaluate three intramuscular injections of 10  $\mu$ g, 25  $\mu$ g, and 50  $\mu$ g doses of RTS,S/AS02A delivered as fractional (0.1 mL, 0.25 mL) or standard (0.5 mL) volumes of the adjuvanted vaccine on a 0-, 1-, and 3-month schedule. All three dose levels of RTS,S/AS02A were found to be safe and well tolerated. There were no serious adverse events related to vaccination. All doses were highly immunogenic for antibodies to CS and HBsAg. Currently, the GSKBio/MVI partnership is sponsoring additional pediatric studies of RTS,S/AS02A in children 1–4 years old. These studies are being conducted in Mozambique by investigators from the Centro de Investigaçao em Saude de Manhica. A Phase 2b proof of concept trial in this population began in mid 2003. Prior to beginning a Phase 3 program in infants, a proof of concept efficacy study will be conducted in a trial designed to demonstrate non-inferiority in regards to immune responses to the Expanded Program of Immunization vaccine antigens when the vaccine is administered (simultaneously but at separate sites) with the diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b vaccine (DTPw/Hib).

**ICC-1132 CS/hepatitis B core particle.** Apovia Inc. (San Diego, CA) in partnership with the MVI and supported by an extramural grant from the National Institute of Allergy and Infectious Diseases, has produced a CS-based particle vaccine that uses the highly immunogenic hepatitis B core antigen (HBcAg) as a delivery platform.<sup>13</sup> ICC-1132, the current clinical candidate, was selected from several preclinical versions on the basis of optimal immunogenicity in mice and other rodents. It includes three NANP repeats plus the

NVDP variant (also a T cell epitope) as well as the “universal” T cell epitope (CS<sub>326-345</sub>) inserted into the HBcAg. During expression in *Escherichia coli*, the proteins self assemble into virus-like particles (VLPs) containing approximately 240 copies of the fusion protein monomer.<sup>14</sup> The particle is engineered so the B cell epitopes are exposed on the surface of the VLP while the T cell epitopes are within the interior. Since the anti-sporozoite antibody response is focused solely to the immunodominant B cell epitopes, there is a 1:1 correlation between the anti-CSP antibody titer and the sporozoite immunofluorescent assay result. Three Phase 1 clinical trials in healthy, malaria-naïve adults are underway. Phase 1 trials in the United Kingdom and Germany have evaluated aluminum hydroxide and Montanide ISA 720 (Seppic SA, Paris, France) formulations. To date, the vaccine appears to be safe and well tolerated. A sporozoite challenge trial in Oxford, United Kingdom was in progress in mid 2003.

**Pf CS 282-383 long synthetic peptide vaccine.** Advances in the manufacturing of long synthetic polypeptides have led to the construction of clinical grade vaccines based on CS and other protein antigens. The University of Lausanne in collaboration with Dictagen, Inc. (Lausanne, Switzerland) is testing a 102-amino acid synthetic peptide prepared using solid-phase synthesis technology. The vaccine incorporates a C-terminal sequence from *P. falciparum* clone NF54 including B cell epitopes, as well as CD8 and CD4 T cell epitopes.<sup>15</sup> Based upon promising preclinical studies in mice and *Aotus* monkeys immunized with formulations alum or Montanide ISA 720, these formulations were evaluated for safety and immunogenicity in malaria-naïve healthy adults. The vaccine formulations were well tolerated and induced antibodies that reacted with intact sporozoites, especially in the volunteers that received the Montanide ISA 720 formulation. The vaccine also promoted a general proliferative response with IFN- $\gamma$  and CD8+ ELISPOT responses detectable following *in vitro* restimulation in some HLA-A\*0201 individuals. A new dose and adjuvant finding Phase 1 study of Pf CS 282-303 formulated with GSKBio AS02A or Montanide ISA 720 adjuvants is currently underway.

**Other pre-erythrocytic stage vaccine candidates.** Experimental and preclinical data have suggested that other pre-erythrocytic stage antigens may be effective vaccine candidates when given alone or when combined with the CS protein. The availability of these candidates has also led to several prime-boost studies in which two different candidates have been administered sequentially in an effort to enhance cell-mediated immune responses. Among the most well-studied candidates are the thrombospondin-related adhesive protein (TRAP or SSP2) and several liver stage antigens, including LSA-1, LSA-3, and exported antigen 1 (EXP-1). LSA-3 is a well-conserved blood-stage as well as pre-erythrocytic antigen, selected by analyses of protective responses in humans that has protected non-human primates, including chimpanzees and *Aotus* monkeys against experimental *P. falciparum* sporozoite challenges.<sup>16</sup> A lipopeptide without adjuvant and a recombinant protein formulated with AS02A are about to enter clinical trials.

**Multi-epitope TRAP.** Investigators at the University of Oxford have constructed novel vaccines consisting of plasmid DNA or recombinant attenuated live viral vectors such as adenovirus, fowlpox (FP9), and modified vaccinia Ankara (MVA) for prime-boost studies.<sup>17</sup> The most advanced of

these studies involve a multi-epitope (ME) string fused to TRAP (of the T9/96 strain). The ME portion contains two B cell, 14 CD8+, and three CD4+ epitopes from six sporozoite and/or liver stage antigens, including CS, LSA-1, and LSA-3. Several Phase 1 and Phase 2a sporozoite challenge studies have been conducted in healthy malaria-naïve adults in the United Kingdom to identify prime-boost vaccination regimens that optimize the cellular-mediated immune response and generate protective immune responses. These regimens induce very high T cell responses in *ex vivo* ELISPOT assays.<sup>18</sup> Some of the tested regimens provided a prolongation of pre-patent period, while other regimens also completely protected a portion of volunteers against heterologous strain sporozoite challenge. The safety of these vaccine candidates was investigated in a series of Phase 1 trials in adults and children in The Gambia.<sup>19</sup> On the basis of good safety profiles, a Phase 2b proof of concept trial of a DNA/MVA prime-boost regimen is being conducted in The Gambia. A series of new FP-9 (fowlpox) and MVA constructs are being manufactured (supported by Wellcome Trust, MVI, and the European Malaria Vaccine Initiative [EMVI]) that are slated for sequential Phase 1 and Phase 2a challenge trials over the next 12 months. In collaboration with GSKBio and the MVI, this group is also evaluating an MVA CS vaccine administered with RTS,S/AS02A in a prime-boost regimen.

**Plasmid DNA vaccines.** The United States Naval Medical Research Center in partnership with the U.S. Agency for International Development (USAID) and Vical, Inc. has developed a vaccine platform that uses combinations of plasmid DNA vaccines that permit targeting of one or more *P. falciparum* antigens.<sup>20</sup> Several single and combination vaccines, including CS alone and CS with SSP2/TRAP, LSA-1, EXP-1, and LSA-3 (MuStDO5), have been tested in Phase 1 and Phase 2a clinical trials. The Phase 1 study of the CS construct when injected as a single vaccine showed it to be safe and well tolerated, but poorly immunogenic for antibody production (none detected), while most subjects developed cell-mediated immune responses, including antigen-specific cytolytic T cell responses and CD8+ ELISPOT IFN- $\gamma$  production.<sup>21,22</sup> Subsequently, a cocktail of all five plasmids was administered alone or with increasing doses of a sixth plasmid encoding human granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjuvant. Although no antibody responses were detected following three doses of DNA, the majority of volunteers developed INF- $\gamma$  responses by ELISPOT. The study showed that the hGM-CSF plasmid was not an effective adjuvant for this vaccine. No protection or delay in prepatent period was observed when these volunteers underwent homologous sporozoite challenge. However, on the basis of stronger post-challenge antibody and T cell responses in vaccinees relative to controls, it was evident that vaccinated subjects had been primed by the DNA immunizations. Interestingly, there was evidence from murine studies of immunologic interference on the part of EXP-1 that may have contributed to suboptimal immunogenicity. Importantly, the Naval Medical Research Center group demonstrated substantial protection in a highly lethal *P. knowlesi* sporozoite challenge in rhesus monkeys using a multistage/multiantigen DNA prime, recombinant pox virus boost. These rhesus studies and other preclinical studies are continuing with the objective of improving the immunogenicity and protection of DNA-based vaccines using a variety of prime-boost strategies. Construc-

tion and manufacturing of multi-gene viral vaccines based on adenovirus-5 and vaccinia (MVA) are underway.

**Peptide CS vaccine for *P. vivax*.** In collaboration with the University of Lausanne (Switzerland), the Malaria Vaccine and Drug Testing Center in Cali, Colombia has recently completed a Phase 1 clinical trial in 69 malaria-naïve healthy adult subjects of three long synthetic peptides derived from the *P. vivax* CS protein. The vaccine was composed of three long peptides representing different fragments of the central and flanking regions of the common type *P. vivax* CS protein, which contain several B, Th, and cytotoxic T lymphocyte epitopes.<sup>23</sup> The first peptide is derived from the amino flank (70 residues) designated as N-peptide, the second represents the carboxyl flank (71 residues) designated as C-peptide, and the third represents the repeat (R) region (48 residues), which contains a trimer built up with a relevant epitope from the repeated blocks linked to a universal T helper cell epitopes from tetanus toxin. Three peptides had been shown to be highly immunogenic in preclinical studies in mice and *Aotus lemurinus* monkeys. Volunteers were randomly distributed in groups of seven subjects and were immunized with either N-, C-, or R peptides formulated in Montanide ISA 720 in increasing doses of 10 µg, 30 µg, and 100 µg by intramuscular injection at 0, 2, and 6 months. Two additional individuals immunized with Montanide ISA 720 alone were used as control for adjuvant. Immunogenicity is being assessed by determination of specific antibodies to each of the three peptides (enzyme-linked immunosorbent assay [ELISA]) and to the native protein (sporozoites) by an indirect fluorescent antibody test. Cellular immune measurements included production of IFN-γ and interleukin-4 in short-term lymphocyte cultures by ELISPOT. The vaccines were safe and well tolerated. Immune responses are being evaluated to assist in the design of Phase 2 trials this year.

## ASEXUAL STAGE VACCINES

A second strategy for the development of a malaria vaccine is to target immune responses against the asexual stage (blood stage) of the parasite. The rationale for this approach is based on the observations that 1) maternal antibodies passively transferred to the fetus may provide a window of protection against clinical malaria, 2) following repeated attacks of malaria, a majority of infected individuals living in endemic areas acquire the ability to control parasite replication to levels below those that result in clinical disease, and 3) hyperimmune globulin prepared from the sera of individuals chronically infected with malaria can eliminate circulating parasites from *P. falciparum*-infected individuals. Although antibodies directed against the parasite are believed to be essential, there is growing evidence from murine models of malaria that cell-mediated mechanisms may be critical to the acquisition of acquired immunity to malaria.<sup>24</sup>

The principal target of current asexual stage vaccine development is the merozoite, the stage that is initially released from the infected hepatocyte and rapidly invades and replicates in circulating red blood cells. Erythrocyte invasion is an energy-dependent, active process that requires the merozoite to contact, adhere, and orient to the red blood cell membrane. During invasion, the membranes fuse, thereby permitting the parasite to become internalized without otherwise damaging

the red blood cell. This complex process is rapid (taking only seconds) and involves a number of parasite proteins that are located on the surface of the merozoite and that are thus transiently accessible to circulating antibodies. The most well-studied antigens include merozoite surface protein 1 (MSP-1), MSP-2, MSP-3, and apical membrane antigen 1 (AMA-1). Antibodies to these molecules are reported to block invasion of merozoites, except for MSP-3, in which they trigger a monocyte-mediated effect. MSP-1, AMA-1, and MSP-3 have been produced as candidate malaria vaccines and have been shown to protect non-human primates from uncontrolled asexual stage parasitemia when administered with Freund's complete adjuvant. Moreover antibodies to MSP-3 can reproducibly transfer protection in a new mouse model of *P. falciparum* malaria.

A major hurdle for the development of malaria vaccines is the marked parasite strain variability associated with many blood stage antigens that require the selection of targets that are relatively conserved or strategies that combine two or more antigens or allelic forms of a single protein. Data in non-human primate models are not required by regulatory authorities, but studies in *Aotus* karyotypes that are susceptible to *P. falciparum* have been useful in concept validation and for promoting clinical development of promising candidate antigens. Their predictive value for selection of protective vaccine candidates remains unclear. Rhesus monkeys have been particularly helpful for assessing immunogenicity and safety because of their size, abundance, and availability of suitable immunologic reagents.

**Merozoite surface protein 1.** One important vaccination strategy is the production of antibodies directed against the carboxy-terminal portion of MSP-1.<sup>25</sup> The Walter Reed Army Institute of Research, in partnership with the USAID, MVI, and GSKBio, has produced a C-terminal *P. falciparum* MSP-1 vaccine as a lyophilized recombinant antigen expressed in *E. coli*. The antigen consists of the 42-kD C-terminal end (392 amino acids) of MSP-1 of the 3D7 clone. The protein is expressed as a fusion protein in which six histidines are added to the N terminal to facilitate purification.<sup>26</sup> The lyophilized antigen is reconstituted in AS02A adjuvant prior to injection. Falciparum merozoite protein 1 (FMP-1)/AS02A was found to be safe and immunogenic in mice, rabbits, and rhesus macaques and induced antibodies against recombinant MSP-1 42-kD antigen as measured by ELISA and against native parasite MSP-1 on infected red blood cells by immunofluorescence assay and invasion inhibition assay. The safety and immunogenicity of FMP-1/AS02A has been assessed in two clinical trials conducted in malaria-naïve individuals. A Phase 1 dose escalation study showed no safety concerns and demonstrated excellent immunogenicity. A subsequent Phase 2a challenge study showed no evidence of protection from infection when FMP-1/AS02A was used alone, nor was it synergistic when co-administered with RTS,S/AS02A. A Phase 1 safety study in malaria-experienced Kenyan adults has been completed successfully, and the vaccine is scheduled to begin Phase 1 studies in Kenyan children 1–4 years old living in a malaria-endemic region of western Kenya.

An important issue is that MSP-1 42kD is polymorphic and exists in nature as two distinct major allelic forms. In *P. falciparum*, the 3D7 and FVO parasite lines express the two major forms of this molecule. Both WRAIR and the National

Institutes of Health (NIH) Malaria Vaccine Development Unit (MVDU) have produced clinical grade antigens [MSP-1(42)] from both allelic forms by expression in *E. coli*. The MVDU approach includes an *in vitro* refolding step to ensure correct structure of the two C-terminal epidermal growth factor-like domains. Rabbit antibodies raised against all of the *E. coli* produced MSP-1(42) antigens blocked invasion of parasites into red blood cells *in vitro*. These blocking antibodies were directed predominantly against the 19-kD portion of the protein. The FVO and the 3D7 forms of MSP-1(42) of the MVDU, as well as a combination of the two antigens formulated with Alhydrogel® (Statens Serum Institute, Copenhagen, Denmark), began Phase 1 trials in 2003.

Vaccine candidates based on the 19-kDa C-terminal fragment of MSP-1 are being developed independently as clinical candidates by teams at the University of Hawaii<sup>27</sup> and the Institut Pasteur.<sup>28</sup> The 19-kD fragment is highly conserved but may not provide as many T cell epitopes as do the longer MSP-1 (42) candidates discussed earlier.

The University of Heidelberg in collaboration with the USAID is developing a full-length MSP-1 vaccine expressed in *E. coli*.<sup>29</sup> The protein is purified using methods that ensure proper folding of the C-terminal domains. Two allelic forms of MSP-1 have been produced for clinical trials and are currently in preclinical formulation studies involving alum and AS02A.

**Apical membrane protein 1.** Apical membrane protein 1 is a complex transmembrane microneme-associated protein containing a signal sequence, an ectodomain comprising three sub-domains formed by eight intra-molecular disulfide linkages, followed by a C-terminal transmembrane region and a cytoplasmic region. Although the exact function of this antigen on the parasite is unknown, AMA-1 is synthesized along the micronemes at the apical end of the merozoite and is relocalized to the merozoite surface during schizogony. Recombinant AMA-1 has been shown to protect animal models (rodents and non-human primates) against parasite challenge, and the preponderance of evidence confirms that protection is antibody-dependent.<sup>30,31</sup>

Walter Reed Army Institute of Research, in partnership with the USAID and GSKBio, have produced a recombinant subunit AMA-1 protein vaccine produced in *E. coli* (FMP2.1). The antigen consists of sequences encoding the "ectodomain" of the AMA-1 membrane protein and comprises 449 amino acids representing the amino acid sequence found in the 3D7 clone of *P. falciparum*. The recombinant protein also contains 18 non-malarial amino acids on the N-terminus including the 6-His tag and 11 non-malarial amino acids on the C-terminus including a second 6-His tag. The lyophilized antigen was adjuvanted with AS02A for Phase 1 studies that began in 2003.

While AMA-1 is an attractive candidate antigen, there is considerable antigenic polymorphism that could limit its impact in the field. Data from animal studies indicate that antisera raised against one form of the molecule that efficiently block the growth of the homologous parasite are less efficient at blocking the growth of heterologous parasites. For this reason, the MVDU has produced clinical grade antigens from two variants of the *P. falciparum* AMA-1: AMA-1(3D7) and AMA-1(FVO).<sup>32</sup> These antigens also differ from those produced by WRAIR in that they contain a slightly longer sequence and they are expressed in *Pichia pastoris*. When

mixed together in equal quantities, the combination, referred to as AMA1-C1, elicits antibodies that block the growth of heterologous parasites as efficiently as the best of its individual components. Clinical trials with AMA1-C1 as a formulation adsorbed on Alhydrogel® began in 2003.

**AMA-1/MSP-1 chimera.** The Second Military Medical University in Shanghai in partnership with the World Health Organization is developing a chimeric fusion of domain III of AMA-1 and the 19-kD portion of MSP-1 called *P. falciparum* chimeric protein 2 (PfCP-2.9). This combination contains conserved portions of both proteins in an attempt to avoid the problems associated with sequence polymorphism. PfCP-2.9 is expressed at high yields in *P. pastoris* and has demonstrated good immunogenicity to both portions of the antigen in mice, rabbits, and non-human primates. Antiserum raised against the PfCP-2.9 can efficiently block *in vitro* parasite growth. Parasite growth inhibition was superior with antisera raised against PfCP-2.9 than to antibody against either of the two domains in isolation. A Phase 1 trial in malaria-naïve healthy adults is underway with a Montanide ISA 720 formulation.

**Merozoite surface protein 3.** The Institut Pasteur in collaboration with the EMVI, has used a long synthetic peptide strategy to construct a polypeptide incorporating regions of MSP-3 covering three human B cell epitopes and three T cell epitopes. A remarkable feature is the full conservation of the MSP-3 critical epitopes. These epitopes were selected on the basis of data indicating that they were targets of human cytophilic antibodies that interact with monocytes to mediate antibody-dependent cell-mediated parasite killing.<sup>33</sup> Since these antibodies are predominantly IgG1 and IgG3, preclinical adjuvant selection included both alum and Montanide ISA 720. A Phase 1 dose ranging study was recently completed and showed that the formulations were safe, well tolerated with alum, and mildly reactogenic with Montanide ISA 720. Immunogenicity data showed unexpectedly that alum was as effective as Montanide ISA 720 for the induction of antibody-dependent cellular immunity-mediating IgG responses in humans and induced antibodies of higher affinity. The antibodies elicited in volunteers were able to inhibit parasite growth both *in vitro* and *in vivo* in the antibody-dependent monocyte-mediated mechanism that is at the origin of the identification of this vaccine candidate. The Institut Pasteur in collaboration with the African Malaria Network Trust has initiated Phase 1b studies in Burkina Faso. A recombinant protein covering the conserved C-terminal region of MSP-3 protein is also under development as a back-up strategy.

**Glutamate-rich protein long synthetic peptide vaccine.** Glutamate-Rich Protein (GLURP) is a blood stage antigen and is present on the parasitophorous membrane of mature schizonts.<sup>34</sup> In a strategy similar to the one used for MSP-3, the Statens Serum Institute and EMVI have developed a 128-amino acid synthetic peptide vaccine using the partial sequence from GLURP. The vaccine contains T cell epitopes as well as conserved antibody-dependent cytotoxic inhibitory epitopes. A Phase 1 trial in normal healthy malaria-naïve volunteers has been recently concluded using vaccine formulated with alum and Montanide ISA720. Local reactogenicity was noted with both preparations including swelling at the contralateral injection site with subsequent injections.

## TRANSMISSION-BLOCKING VACCINES

Preclinical studies conducted over the past decade have clearly demonstrated that antibodies directed against several sexual stage antigens are capable of preventing the development of infectious sporozoites in the salivary glands of *Anopheles* mosquitoes, thereby suggesting that so-called transmission blocking vaccines (TBV) might be an effective weapon against malaria. In practice, a population with sufficient immunization coverage with TBV could break the cycle of parasite infection and transmission associated with their local vectors. By their nature, TBVs do not confer protection to individuals and thus vaccination coverage will likely need to be widespread and sustained. Process development for several leading TBV candidates has proven to be very challenging. Sexual stage antigen targets have proven to be complex proteins with precise molecular folding requirements and formulation of adequately immunogenic vaccines has been difficult.<sup>35</sup>

***Plasmodium vivax* s25 and *P. falciparum* s25.** Satisfactory expression refolding and purification of highly characterized clinical grade candidate antigens, including the *P. vivax* antigen Pvs25 and its *P. falciparum* ortholog Pfs25, has recently been achieved by the MVDU intramural of the NIH. Pvs25 produced in *S. cerevisiae* elicits antibodies in mice, rabbits, and non-human primates that efficiently block transmission in membrane feeding assays. A formulation adjuvanted with aluminum hydroxide is currently in a Phase 1 clinical trial. Similar preclinical data have been obtained with Pfs25 expressed in *P. pastoris*, setting the stage for a Phase 1 trial with this vaccine candidate. Future clinical development is likely to involve combinations of these and other TBV antigens.

## CONCLUSIONS

Over the past five years, substantially increased funding from public-private partnerships has focused greater resources on the GMP production of promising vaccine candidates and conduction of Phase 1 and Phase 2 studies. The result has been a remarkable increase in the quality and number of candidate vaccines entering clinical trials and considerable optimism that major progress is being made. Indeed, there are currently three vaccine candidates (RTS,S/AS02A, MVA-ME TRAP, and MSP-1/AS02A) being studied in target (pediatric) populations in field settings. The good news is that many new North-South collaborations have been initiated and candidate vaccines for each of the major stages of the parasite life cycle are being evaluated in clinical trials. There is increasing involvement on the part of industry that brings crucial preclinical experience, manufacturing know-how, and clinical development capabilities that will significantly increase the chances of success. The not-so-good news is that there remains a critical need for investigators in developing countries with strong clinical development skills. More importantly, there are clearly insufficient financial and personnel resources identified to date to support the complete development of the most promising candidates. Indeed, the clinical development of one such candidate that underwent promising field studies in Papua New Guinea was recently abandoned largely due to lack of sufficient financial and industrial resources.<sup>36</sup> Increasingly complex vaccination approaches are being used, including prime-boost strategies in-

volving two (or more) vaccines and pose regulatory challenges. The way forward will require that researchers continue to refine target product profiles to be very clear on where and how the vaccines will be used and that there be greater public-private partnership involvement in these discussions. The reality that investments will be greatest at the final stages of clinical development (i.e., pivotal licensure trials) must be recognized. The risks associated with the decision to support such trials must be balanced by carefully defined, quantifiable Go/NoGo endpoints. Malaria vaccine development is at an exciting and very promising stage; it is essential that the momentum achieved to date be sustained in the years ahead.

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